

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

#159

RENEWED
Application For Research Grant

(Refer to #74, activated
June 1, 1955 and renewed
June 1, 1956)

Date: April 22, 1957

1. Name of Investigator: **Cecilie Leuchtenberger, Ph.D.**

2. Title: **Associate Professor of Cytology and Biology**

3. Institution & Address:
**Institute of Pathology
Western Reserve University
2085 Adelbert Road
Cleveland 6, Ohio**

4. Project or Subject:
A correlated histological, cytological and cytochemical study of the tracheo-bronchial tree from mice exposed to cigarette smoke.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aims

The purpose of this project is the continuation and extension of the histological, cytological and cytochemical studies of the tracheo-bronchial tree from mice exposed to cigarette smoke. Previous studies done in our laboratory on the same problem have revealed distinct histological, cytological and cytochemical changes in the tracheo-bronchial tree from mice exposed to cigarette smoke. The experimental conditions and the results obtained so far have been described in progress reports sent to the Tobacco Industry Research Committee (see especially the Progress Report of June 1956 to March 1957). Furthermore, a paper entitled: "Quantitative Determination of Nucleoproteins by Microspectrophotometry and of Dry Mass by Interference Microscopy in Bronchial and Lung Cells from Mice Exposed to Cigarette Smoke" has been presented by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger and Mr. Paul F. Doolin at the American Association for Cancer Research in Chicago on April 12, 1957.

There are several main problems which warrant further thorough investigation:

(1) A follow-up of the early cytochemical and histopathological changes, especially of the atypical hyperplasia, the squamous cell metaplasia and "carcinoma in situ". It seems of importance to find out whether these alterations of the epithelium are reversible, stationary or may lead to invasive

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cancer. Consequently, mice will be exposed for varying periods to cigarette smoke and the sequential changes in the tracheo-bronchial tree will be studied during the whole life span of the mice. Such experiments may also yield information of a more general nature as to the significance of "carcinoma in situ" for the evolution of cancer.

(2) Since inflammatory processes such as bronchitis and peribronchitis were observed very frequently in the mice exposed to cigarette smoke, the question arises whether the atypical epithelial changes may not just be an expression of these bronchitic alterations. In order to obtain an answer to this question, it would be desirable to induce bronchitis and peribronchitis in mice by means other than cigarette smoke.

(3) In order to test the theory proposed by some scientists that cigarette smoke acts as a carcinogen, it would be of significance to expose mice to a known carcinogen, such as benzpyrene, and then study the sequential histological, cytological and cytochemical changes exactly as it is done after exposure to cigarette smoke.

5. B. Material and Methods

The material and methods will be essentially the same as in the previous studies. However, in order to permit a larger scope of the studies, greater number of mice will be utilized and a second smoking machine will be built and used. Following a suggestion of Dr. Paul Kotin of the University of Southern California, the second smoking machine will be modified in such a way that the mice can be exposed to intermittent cigarette smoke. For the cytochemical analysis microspectrophotometry and interference microscopy will be utilized and it is also hoped that correlating with these methods some electron-microscopic studies can be done.

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6. Budget Plan:

Salaries	17137.50
Expendable Supplies	1300.00
Permanent Equipment	*1 1000.00
Overhead	2990.62
Other	500.00
Total	\$ 22928.12

*1 Specially designed smoking machine.

*2 Travel (attendance at scientific meetings; consultations.)

7. Anticipated Duration of Work: Three to five years.

8. Facilities and Staff Available:

The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. C. Leuchtenberger) and suitable animal quarters in the Institute of Pathology, Western Reserve University. The cytochemical laboratories are well-equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are: (1) Four microspectrophotometric apparatuses; (2) 1 one ultraviolet microspectrophotometer; (3) six research microscopes; (4) two phase microscopes; (5) two photomicrographic setups; (6) one Beckman spectrophotometer; (7) one freeze-drying apparatus for the fixation and embedding of tissues; (cont'd next page)

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10. Additional Information (Including relation of work to other projects and other sources of supply):

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Signature /s/ Cecilia Leuchtenberger, Ph.D.
Director of Project Associate Professor of Cytology
and Biology

/s/ S. F. Agnew, Comptroller
Business Officer of the Institution

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(8) two interference microscopes; and (9) tobacco research laboratory including newly designed smoking machine. However, the animal quarters will have to be extended to provide sufficient facilities for expanding the number of experiments now deemed necessary to pursue these types of investigations. Furthermore, we have one especially designed smoking machine, but again, to expand the investigations, at least one more machine is needed. There is also a team of workers (professional and technical) comprising cytochemist, pathologists and research assistants.

No. 9 - Additional Requirements

Personnel

1. Experienced cytologist at Ph.D. level	\$6000	+	2½ Soc. Sec.	=	6094.50
2. (2) Research technicians trained in histological procedures and animal experimentation	\$7200	+	" "	=	7362.00
3. Part time secretary and research librarian	\$1200	+	" "	=	1227.00
4. Animal caretaker and glassware washer	\$2400	+	" "	=	2454.00
		<hr/>			\$ 17137.50
			\$ 16800		

Expendable supplies

1. Animals and animal food (approximate estimation)	\$ 500.00
2. Cigarettes and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	<hr/> \$ 800.00
	\$ 1300.00

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